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MicroRNAs in Barrett's esophagus and esophageal adenocarcinoma

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<Title>

MicroRNAs in Barrett's esophagus and esophageal adenocarcinoma

<Short Title>

miRNAs and esophageal neoplasms

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<Summary>

The molecular genetics of Barrett's esophagus (BE) and its evolution to esophageal adenocarcinoma (EAC) have been widely studied; however, the molecular mechanism of BE–EAC carcinogenesis has not been completely understood. MicroRNA (miRNA) is now essential to understand the molecular mechanism of cancer progression. Recent findings include the following: firstly, miRNA expression profiles can distinguish between BE and EAC; secondly, miR-196a is upregulated in EAC tissues targeting annexin A1, thereby exerting antiapoptotic effects and contributing to EAC cell survival; miR-196a may also constitute a good biomarker of progression during BE–EAC carcinogenesis; and thirdly, The miR-106b-25 polycistron is activated by genomic amplification and is involved in esophageal neoplastic progression and proliferation via the suppression of two target genes, p21 and Bim.

<Introduction>

Barrett's esophagus (BE) is a metaplastic condition in which the normal squamous epithelium of the lower esophagus is replaced by a small intestinal-like columnar lining [1 A.J. Cameron, Management of Barrett's esophagus, Mayo Clin Proc 73 (1998), pp. 457–461. View Record in Scopus | Cited By in Scopus (44)1]. BE starts as a noncancerous metaplastic lesion and may progress to low-grade dysplasia (LGD), high-grade dysplasia (HGD), and finally to frank esophageal adenocarcinoma (EAC). Although the molecular basis of BE and its evolution to EAC have been widely studied [[2], [3], [4] and [5]], molecular mechanisms of BE–EAC carcinogenesis have not yet been completely elucidated. Improved biomarkers and a better understanding of the mechanisms of this neoplastic progression are therefore eagerly awaited.

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MicroRNA (miRNA) is a newly discovered class of abundant, small, naturally occurring RNAs. miRNA inactivates multiple target genes by sequence-specific binding-mediated destabilization of mRNA or inhibition of translation. Although the first miRNA, lin-4, was discovered in 1993 and the second miR, let-7, in 2000 in Caenorhabditis elegans, only recently has the study of this class of small regulatory RNAs in humans become more widespread. Experimental evidence accumulating in recent years has led oncologists to speculate that unrevealed molecular actors, particularly noncoding RNAs previously been classified as ‘junk,’ play important roles in carcinogenesis. In fact, as much as 70% or more of the genome has been found to be transcribed in mammals [6]. Thus, it is a natural extension of this logic to explore novel noncoding RNA-based mechanisms underlying cancer-related gene dysregulation. miRNAs have been intensively studied in cancer research, and these molecules are predicted to control as much as 30% of all gene expression alteration [7]. miRNA regulatory mechanisms involved in cancer progression may be viewed as previously missing pieces of a puzzle that are now essential to mapping the molecular landscape of cancer progression. Experimental evidence has contributed to the understanding of molecular mechanisms of miRNAs not only in cancers in general, but also specifically in BE and EAC in particular.

In this review, we highlight three issues of current relevance to this field and discuss firstly, recent findings regarding the taxonomy, biogenesis, and functions of miRNA; secondly, some of the latest findings on the molecular mechanisms of miRNA involved in BE and EAC; and thirdly, the therapeutic–clinical potential of miRNAs in BE–EAC neoplastic progression.

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Surveillance of BE and EAC.

BE is a premalignant condition that predisposes individuals to the development of EAC, one of the most rapidly increasing cancers in developed nations [8]. Estimates of the prevalence of BE range from 0.9% to 4.5% [[9] and 10 W.K. Hirota, T.M. Loughney, D.J. Lazas, C.L. Maydonovitch, V. Rholl and R.K. Wong, Specialized intestinal metaplasia, dysplasia, and cancer of the esophagus and esophagogastric junction: prevalence and clinical data, Gastroenterology 116 (1999), pp. 277–285. Abstract | View Record in Scopus | Cited By in Scopus (308)[10]]. A recent study in Sweden revealed that BE had a prevalence of 1.6% (of 21 610 patients) [11], implying that more than three million individuals may harbor BE in the United States alone [12]. Among these patients, EAC susceptibility is a life-threatening long-term sequela. The incidence of EAC in BE is estimated 1.8 per 1000 person-years, 30-fold higher than in the general population [13]. It is recommended that BE patients undergo surveillance endoscopy. However, histology-based surveillance is insufficient because of poor reliability of the histological grading of BE [14•] and poor specificity to predict the progression to cancer [15]. Moreover, although EAC is frequently accompanied by Barrett's metaplasia, only approximately 5% of patients presenting with EAC have an antecedent diagnosis of Barrett's metaplasia [16]. Thus, the majority of patients presenting with EAC will therefore not benefit from refinements to endoscopic surveillance programs for Barrett's metaplasia.

The molecular genetics of BE and its evolution to EAC have been widely studied. However, this process still needs to be better understood in order to provide firstly, clues to biologic pathways underlying BE-associated

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neoplastic transformation; secondly, biomarkers of early cancer detection, disease progression, or ultimate prognosis; and thirdly, therapeutic strategies to prevent and treat this disease process.

Taxonomy, biogenesis and functions of the miRNA

Various noncoding small RNAs with distinctive characteristics have been found. It is widely believed that there are three categories of noncoding small RNAs, based on their mechanism of biogenesis and the type of Argonaute protein they are associated with. These three categories are miRNAs, endogenous small interfering RNAs (endo-siRNAs or esiRNAs), and Piwi-interacting RNAs (piRNAs) [17•]. miRNAs are a class of abundant, approximately 22-nucleotide, noncoding small RNAs that mediate post-transcriptional regulation of their cognate target mRNAs. Figure 1 illustrates the biogenesis of miRNA. Nascent miRNA (pri-miRNA) is generated by RNA polymerase II [18] or III [19] and processed within the nucleus into approximately 70-nucleotide incomplete stem–loop RNA (pre-miRNA) by the RNase III endonuclease Drosha and DGCR8 (Drosha/DGCR8 complex) [20]. Exportin 5 transports pre-miRNA from the nucleus to the cytoplasm. The cytoplasmic RNase III Dicer then cuts pre-miRNA into a 19–22-base long RNA duplex [21]. RISC (the RNA-induced silencing complex) incorporates the short RNA duplex and activates one strand of RNA (mature miRNA) while the other strand is degraded [17•]. The biological role of miRNA is to inactivate single or multiple target genes by sequence-specific binding-mediated mechanisms. **Figure 1**. Biogenesis of the miRNAs. Nascent miRNA (pri-miRNA) is generated by RNA polymerase II or III and processed within the nucleus into approximately 70-nucleotide incomplete stem–loop RNA (pre-miRNA) by the RNase III endonuclease Drosha and DGCR8 (Drosha/DGCR8 complex). Exportin 5 transports pre-miRNA from the nucleus to the cytoplasm.

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The cytoplasmic RNase III Dicer then cuts pre-miRNA into a 19–22-base long RNA duplex. RISC (the RNA-induced silencing complex) incorporates the short RNA duplex and activates one strand of RNA (mature miRNA) while the other strand is degraded. The biological role of miRNA is to inactivate single or multiple target genes by sequence-specific binding-mediated mechanisms.

MiRNA and cancer

Cancer-specific miRNAs and fingerprints have been identified in every type of tumor analyzed [[22••] and [23••]]. To date, numerous miRNAs have been identified as tumor-related and can be categorized in two groups based on their functional relevance. miRNAs that have oncogenic effects/roles are categorized in oncogenic miRNAs (oncomiRs), while miRNAs that have tumor-suppressive effects/roles are categorized in tumor-suppressive miRs (ts-miRs). In normal cells, ts-miRs are highly expressed and downregulate the expression of oncogenic proteins, whereas in tumor cells, ts-miRs are silenced, leading to the upregulation of oncogenic proteins. Conversely, oncomiRs are upregulated in tumor cells, downregulating the expression of tumor-suppressive proteins. For example, the miR-17–92 cluster has been characterized as oncogenic (oncomiRs) [24 L. He, J.M. Thomson, M.T. Hemann, E. Hernando-Monge, D. Mu, S. Goodson, S. Powers, C. Cordon-Cardo, S.W. Lowe and G.J. Hannon et al., A microRNA polycistron as a potential human oncogene, Nature 435 (2005), pp. 828–833. Full Text via CrossRef | View Record in Scopus | Cited By in Scopus (719)24] and miR-21 also has been found to be upregulated in various types of cancers downregulating tumor suppressor genes such as tropomyosin 1 (TPM1), programed cell death 4 (PDCD4), and tissue inhibitor of metalloproteinase 3 (TIMP3)

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[25•], while let-7 represses Ras [26] and miR-15a and miR-16-1 repress Bcl-2 [27], thereby acting as tumor suppressors (ts-miRs).

MiRNA in BE and EAC

To date, a few miRNA studies have been reported in BE and EAC; however, experimental evidence has steadily elucidated the molecular mechanisms underlying BE and EAC. miRNA expression profiles in BE and EAC were first reported by Feber et al. [28•]. Studying surgical specimens of 10 squamous cell carcinomas of the esophagus, 5 BEs, 1 HGD and 10 EACs as well as 5 normal squamous epithelia (NSE), they successfully demonstrated that miRNA expression patterns discriminated between tissue types. Since miRNA expression profiling has shown to provide more accurate classification of tissue and tumor types than global messenger RNA expression profiles [[29] and [30]], miRNA expression profiling is now a promising method to identify key effectors that play important roles in BE–EAC carcinogenesis. In fact, Feber et al. discovered interesting results in this context. Among the upregulated and downregulated miRNA lists they generated, miR-192, miR-194, miR-21, and miR-93 were progressively upregulated during progression from NSE to BE and finally to EAC. miR-192 is upregulated in lung cancer and exerts proliferative effects [31] and is also overexpressed in colon cancer with effects on cell cycle progression [32], suggesting that miR-192 possesses oncogenic (oncomiR) properties. miR-21 is also a well-characterized oncomiR that is upregulated in a number of different cancers [[25•], [33] and [34]]. In contrast, one group revealed that miR-205 was downregulated in both BE and EAC versus NSE, implying tumor-suppressive (ts-miR) properties for this miR. Interestingly, this finding is compatible with our own recent study [35••], which comprises the latest report regarding miRNA dysregulation

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in BE and EAC. miR-205 has also been reported to be downregulated in prostate cancer, where it has been linked to the targeting of PKC epsilon [36] as well as breast cancer where it targets HER3 as well as ErbB3 and VEGF-A [[37] and [38]]. Childs et al. reported that miR-205 (and let-7d) downregulation, was significantly associated with poor survival in head and neck cancer [39], suggesting potential application as prognostic indicators. In view of all the above findings, miR-205 may constitute a good biomarker for BE as well as EAC.

Luthra et al. reported that miR-196a was upregulated in EAC tissues, targeting annexin A1 (ANXA1) [40•]. miR-196a downregulates ANXA1, consequently exerting antiapoptotic effects and contributing to EAC cell survival. This group further pursued this finding, reporting that miR-196a is a good potential biomarker of neoplastic progression in BE, and demonstrating the stepwise upregulation of miR-196a expression during the BE–dysplasia–EAC sequence [41••].

Finally, our own group recently reported several miRNA-related mechanisms involved in BE and EAC [35••]. In our initial approach, miRNA microarrays were performed to identify upregulated or downregulated miRNAs in BE-associated lesions. Strikingly, we discovered that miR-25, miR-93, and miR-106b, all of which form a polycistron on chromosome 7q22.1 (the miR-106b-25 polycistron), were progressively activated at successive stages of esophageal neoplasia from normal epithelium to BE and finally to EAC. Upregulation of the miR-106b-25 polycistron was also associated with genomic amplification and overexpression of MCM7 (the mother gene containing this polycistron), potentially because of de novo genomic DNA copy number variation involving the MCM7 locus at chromosome 7q22.1. Moreover, the miR-106b-25 polycistron exerted potential

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proliferative, antiapoptotic, and cell cycle-promoting effects in vitro and tumorigenic effects in vivo. We found that miR-93 and miR-106b targeted and inhibited CDKN1A (p21), whereas miR-25 targeted and inhibited Bcl-2-like protein 11 (BCL2L11, Bim). The miR-106b-25 polycistron thus represents a likely oncomiR involved in BE–EAC carcinogenic evolution, as well as in other human cancers [[42] and [43]], and may act via the regulation of two specific target genes, p21 and Bim. Representative miRNA expression levels in BE and EAC and their potential target transcripts are shown in Table 1.

Therapeutic/clinical application of miRNAs

OncomiRs are presumed to function by downregulating tumor suppressor genes. OncomiRs also have the potential to serve as molecular therapeutic targets, since their inhibition should result in increased levels of tumor suppressor proteins [[35••] and [40•]]. Conversely, it can be postulated that re-introduction of ts-miRs into tumor cells will result in the downregulation of target oncogenes, causing tumor suppression [[37], [44] and [45]]. At present, there are no reports of the use of miRNA for anticancer therapy in the clinical field. However, miRNA-based gene therapy provides an attractive antitumor approach for integrated cancer therapy [46•]. One advantage of miRNAs over siRNAs/shRNAs is that miRNAs target multiple genes simultaneously, powerfully inactivating entire pathways at once. This effect contrasts markedly with the unpredictable and undesirable off-target effects of siRNAs/shRNAs. Emerging reports on miRNAs in BE and EAC have suggested that miRNAs are promising in categorizing BE-associated lesions (including dysplasia) into subgroups, stratifying the risk of susceptibility to developing EAC, diagnosing EAC, and developing future therapeutic targets in BE and EAC.

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<Conclusions>

Recent gains in our knowledge of miRNA expression and its role in BE and EAC have considerably advanced our understanding of the molecular mechanisms underlying BE-associated neoplasia. miRNA profiling shows promise as an accurate diagnostic tool, and BE-specific or EAC-specific miRNAs may eventually constitute useful biomarkers as well as therapeutic targets.

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<Conflicts of Interest>

The authors have no conflicts to disclose.

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In this review, the authors focus on miRNA-based treatment for cancers, summarize the delivery systems used in experimental and preclinical research, and consider the safety and toxicity of miRNA therapy. MiRNA-based gene therapy provides an attractive anti-tumor approach for integrated cancer therapy.

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<Figure legends and Table notes>

Figure 1. Biogenesis of the miRNAs. Nascent miRNA (pri-miRNA) is generated by RNA polymerase II or III and processed within the nucleus into approximately 70-nucleotide incomplete stem-loop RNA (pre-miRNA) by the RNase III endonuclease Drosha and DGCR8 (Drosha/DGCR8 complex). Exportin 5 transports pre-miRNA from the nucleus to the cytoplasm. The cytoplasmic RNase III Dicer then cuts pre-miRNA into a 19-22-base long RNA duplex. RISC (the RNA-induced silencing complex) incorporates the short RNA duplex and activates one strand of RNA (mature miRNA) while the other strand is degraded. The biological role of miRNA is to inactivate single or multiple target genes by sequence-specific binding-mediated mechanisms.

Table 1. Data were from the following references: [28] [35] [41]. # indicates stepwise expression in Barrett's esophagus and esophageal adenocarcinoma from normal squamous epithelium

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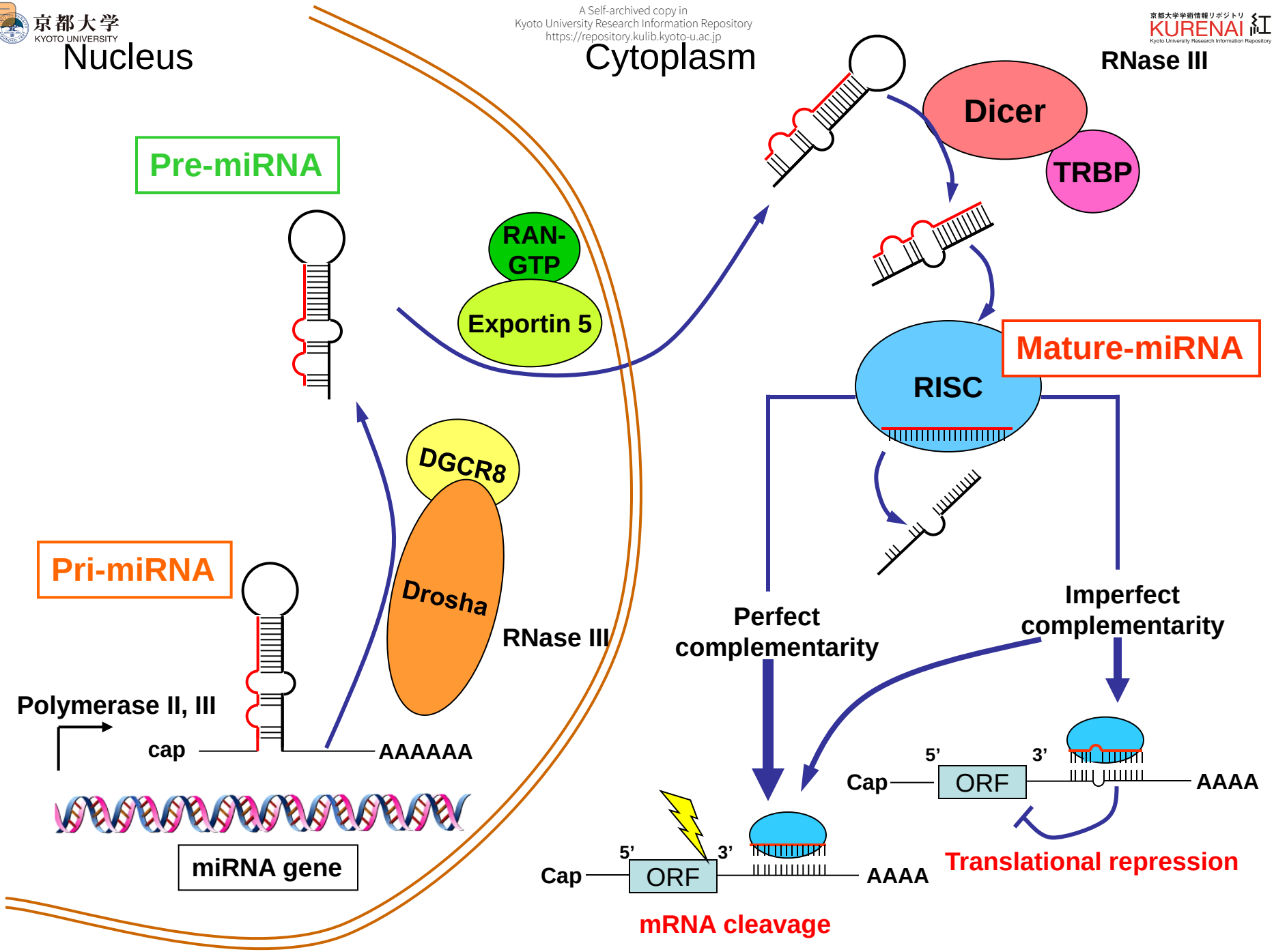

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Upregulated miRNAs

Barrett's Esophagus	Esophageal Adenocarcinoma	Potential Target Transcripts	Functions
miR-21	miR-21	BMPRII, LRRF1P1, BTG2, PTEN, PDCD4, TM1, MARCKS, TIMP3	Cell cycle, Cell proliferation, Apoptosis
miR-192#	miR-192#	DHFR, CDC7, LMNB2, MAD2L1, CUL5	Cell cycle, Cell proliferation
miR-194#	miR-194#	EP300	Metastasis
miR-200c	miR-200c	ZEB1, ZEB2, TUBB3	Epithelial-mesenchymal transition (EMT)
miR-196a#	miR-196a#	ANXA1, KRT5, SPRR2C, S100A9	ANXA1, KRT5, SPRR2C, S100A9
miR-25#	miR-25#	BCL2L11	Apoptosis
	miR-93	CDKN1A	Cell cycle
	miR-106b	CDKN1A	Cell cycle

Downregulated miRNAs

Barrett's Esophagus	Esophageal Adenocarcinoma	Potential Target Transcripts	Functions
	miR-205	HER3, PRKCD, VEGFA	Cell proliferation, EMT
	miR-203	ABL1, TP63	Cell proliferation
	miR-27b	ST14, CYP1B1	Cell proliferation, Cell migration, invasion, Drug metabolism
miR-125b	miR-125b	CYP24, ERBB2, ERBB3	Cell proliferation
	miR-100	FRAP1, RPTOR	CMV replication
	miR-19b	E2F1, MYC, BCL2L11, CDKN1A, HIF1	Cell proliferation, Apoptosis, Cell cycle